Novel Therapeutics for Enteric Diseases
Workshop Summary

Robert H. Hall, Ph.D.
Enteric and Hepatic Diseases Branch
Division of Microbiology and Infectious Diseases
National Institute of Allergy and Infectious Diseases
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Bethesda, MD 20892

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Summary
On September 22, 2005, a group of researchers studying enteric infections met in Bethesda, MD at the invitation of the Enteric and Hepatic Diseases Branch of the National Institute for Allergy and Infectious Diseases. The objective of the meeting was to discuss enteric disease, summarize the current understanding of its burden and mechanisms, and review possible therapeutic approaches to its mitigation. The workshop concluded that recent research has opened several important opportunities for new products, and that mitigating enteric disease will entail integrating multidisciplinary research with further drug development.

Burden of Disease
The burden of diarrheal disease by some measures has significantly declined in the years since oral rehydration therapy (ORT) became the global standard of care. More detailed analyses of the data, however, indicate that while deaths from acute diarrhea have declined somewhat in the last forty years, the total number of cases has increased significantly. Previous estimates based on disability adjusted life years (DALYs) attributed 5% of the burden of disease to morbidity (incapacitation during the acute phase) and 95% to mortality. A recent re-assessment showed the reverse, that 95% of the overall burden of disease was morbidity and 5% was mortality. The true burden of enteric infections is therefore approximately an order of magnitude greater than previously estimated. Without question, the syndrome of diarrhea represents a major challenge to child health globally, matching and exacerbating the blights of tuberculosis, HIV/AIDS, and malaria.

Problems with Present Therapies
Present standards of care depend on the use of ORT and effective antimicrobials. It is the increasing presence of antimicrobial resistance, limited therapeutic options for many parasitic and viral infections, and the worrisome under-use of ORT that has led to the current crisis. Drug resistance can be fought either with vaccines or novel therapeutics. The potential for enteric vaccines was addressed previously. The potential for new therapeutics was the topic for the present workshop.

Gaps in Knowledge

Recent years have seen a welcome shift in drug discovery away from its empirical origins toward a more rational, targeted approach, and several promising drug candidates are undergoing pre-clinical evaluation and clinical trials. In considering novel therapeutic approaches to mitigating diarrheal disease, two options are available, attacking the virulent agent directly, or addressing the physiological insult with a remedial therapy. Both approaches require an integrated understanding of molecular pathogenesis and host response. The overarching aim should be to develop more effective therapies that preserve normal intestinal microflora and do not drive the emergence of antimicrobial resistance. Targeted, narrow-spectrum therapy requires up-to-date epidemiological information and specific diagnostics to keep pace with changing patterns of emerging enteric diseases both domestically and abroad, such as drug resistant pathogens, *Clostridium difficile,* the microsporidia, enteric viruses, and co-infections. Likewise, developing drugs that mitigate or repair the damage to the host depend on a deeper understanding of pathogenesis, inflammation, fluid loss, and tissue damage during infection.

New Approaches to Antimicrobial Therapy

Several intriguing candidates are presently in the drug development pipeline. The sources, concepts, and mechanisms of these potential drugs illustrate the extraordinary range of opportunities that have emerged from a robust program of basic research.

Nitazoxanide (Romark Laboratories) is a structural analog of thiamine pyrophosphate and may be the first of a new class of antimicrobial drugs that inhibits the function of a cofactor. Several parasites and other pathogens utilize pyruvate:ferredoxin oxidoreductase (PFOR) to convert pyruvate and CoA to acetylCoA, a reaction accomplished by pyruvate dehydrogenase in humans. Nitazoxanide inhibits PFOR by a mechanism that is not fully understood. Clearly an important line of research is to better understand the activity of nitazoxanide and optimize its effectiveness against a range of pathogens that utilize PFOR.

Among the new antibacterial compounds, rifaximin (Salix Pharmaceuticals) represents an approach to develop an orally administered, non-absorbable drug with activity in the gut lumen against bacterial enteropathogens. Classically, bacteriocidal or bacteriostatic compounds select for drug resistance and adversely affect the intestinal flora, but so far the expected antimicrobial resistance has not become a problem, and rifaximin does not appear to eliminate the normal flora as thoroughly as ciprofloxacin, for example.

Targeting the regulatory elements that control bacterial pathogenesis is an exciting new approach to develop narrow spectrum therapeutics. Virstatin acts by inhibiting cholera toxin gene expression, and has been shown in animal models to mitigate diarrheagenesis. Virstatin does not need to be absorbed systemically by the host to be effective and it is unlikely to select for drug resistance or attenuate the intestinal flora.

The bacterial Mar system provides potential targets among the highly conserved AraC family of DNA binding proteins. Over 1,000 orthologs of AraC proteins have been identified in both gram-negative and gram-positive pathogens. Paratek Pharmaceuticals has developed small molecule inhibitors for the MarA orthologs of *E. coli* and *Yersinia,* and efficacy has been shown in preliminary animal studies. As with virstatin, MarA inhibitors are unlikely either to select for drug resistance or adversely impact the normal intestinal flora.

Other Approaches to Prevention and Cure

Therapeutics that address inflammatory and other host responses are unlikely to affect the intestinal microflora or drive drug resistance. Promising experimental anti-inflammatory compounds include adenosine and adenosine analogs.

The efficacy of nursing at preventing enteric infections among infants is legendary. A significant element of protection apparently is mediated by human milk glycans, many of which have a significantly inhibitory effect on enteric pathogens *in vitro*. Improved methods are facilitating the synthesis and manufacture of increasingly complex glycans, and further study in this area may lead to a new field of valuable therapeutics.

At the other extreme lies phage therapy, a technology born over 100 years ago, but also presently searching for a place in the modern armamentarium. Phage therapy offers the theoretical advantage of exquisite specificity and an ability to lyse immense populations of bacteria with great speed. Although resistance to phage can emerge quickly, phage do offer a possible avenue to attack otherwise untreatable drug resistant infections. Recent advances in the understanding of phage biology have raised the possibility of engineering recombinant particles with optimized features, and further research may uncover novel and efficient pathways to bacterial lysis. Whether enteric infections represent the best arena for demonstrating the power of therapeutic phage is an open question.

The normal intestinal flora plays a critical role both in the prevention of disease and in re-establishing the health of the convalescent, yet it remains a poorly understood ecosystem. Metagenomics studies will increasingly probe the interdependence of the human host and the enteric flora. Discovering the elements of a healthy intestinal ecology may provide additional data supporting the use of specific probiotic products.

Oral Rehydration Therapy

Few would dispute that ORT stands as one of the most brilliantly simple and effective contributions to medicine. The checkered history of its conception, refinement, and adoption has been described in detail, and there is an exciting future for this elegant therapy. Current formulations of ORT should be more widely advocated, especially to the elderly, and abundant packaged salts should be pre-placed in patient care settings. Improvements in formulation are possible with carbohydrate bases that are more readily absorbed or more locally available. In some cases, oral rehydration formulations could be supplemented with therapeutics, amino acids, or micronutrients to mitigate disease, enhance intestinal repair, expedite recovery, and help conserve resources in epidemic settings.

Anti-Secretory Compounds

The best understood mechanism of diarrheagenesis involves activation of the intestinal cystic fibrosis transmembrane conductance regulator (CFTR), an ABC transporter spanning the enterocyte apical membrane. The key enterotoxins produced by both enterotoxigenic *Escherichia coli* (ETEC), and *Vibrio cholerae* activate CFTR through a complex chain of events that results in chloride efflux, loss of sodium ions to maintain electroneutrality, followed by the loss of water to maintain osmotic balance. In addition to cholera and ETEC diarrhea, many researchers predict that CFTR activation underlies the mechanism of other secretory diarrheas for which no mechanism has yet been determined. CFTR is a promising therapeutic target and it can be effectively inhibited at its intracellular face by orally-administered absorbed drugs or alternatively from its gut lumenal face by non-absorbed drugs.

The natural drug product crofelemer (Napo Pharmaceuticals) extracted from the bark latex of the tree *Croton lechleri* is a non-absorbed mixture of polyphenolic compounds that bind the luminal face of CFTR and inhibit CFTR-mediated chloride secretion. Clinical studies involving 1,400 patients have established that the drug is well tolerated and effective in mitigating travelers' diarrhea, and chronic HIV/AIDS-associated diarrhea of unknown etiology. Crofelemer is not an antimicrobial, and therefore does not drive the emergence of drug resistance, it does not inhibit gut motility, and therefore does not cause constipation or rebound diarrhea, and it is not systemically absorbed, reducing the potential for adverse drug interactions and toxicity.

Two early-stage CFTR inhibitors are presently under development as drug candidates by ActivePass Pharmaceuticals, of Vancouver, Canada. The thiazolidinone compound CFTR-172^{inh} and a series of glycine hydrazides inhibit CFTR and mitigate diarrhea in animal models by binding respectively the interior (intracellular) and exterior (lumenal) faces of CFTR. Future work with these promising compounds includes completion of preclinical testing in preparation for clinical evaluation.

Future Management of Enteric Infections

Presently, managing enteric infections involves rehydration and antimicrobial therapy. Even in responsive cases, severe intestinal damage such as inflammation and the effacement of intestinal microvilli may occur during the 36 hours it takes for antimicrobial drug activity to eliminate a sensitive pathogen. New findings have raised the prospects of adding intestinal repair, anti-diarrheal drugs, and novel narrow-spectrum antimicrobials to the physician's armamentarium, which together could greatly expedite recovery, reduce the duration of hospitalization, reduce the demands for rehydration fluids, and reduce the pressure for development of antimicrobial resistance.

The opportunities in developing new approaches to address enteric disease stem from robust research programs into both the pathogen and the host. Cross-fertilization of ideas is increasingly possible, but there remains a great deal to be understood about the basic mechanisms of diarrheagenesis during enteric infection.

The workshop concluded that new therapeutics for enteric diseases represent an area where continued attention by NIAID could have a major impact on what has become a devastating public health problem. The combination of ORT, anti-diarrheal drugs, antimicrobials and vaccines should be seen as a multi-pronged response to a multifaceted problem.